

ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY: A REVIEW

Patil V. P.^{1*}, Tathe R. D.¹, Devdhe S. J.², Angadi S. S.² and Kale S. H.³

¹Department of Pharmaceutical Analysis, Yash Institute of Pharmacy, Aurangabad, Maharashtra, India

²Department of Pharmacology, Yash Institute of Pharmacy, Aurangabad, Maharashtra, India

³Department of Pharmaceutical Chemistry, Yash Institute of Pharmacy, Aurangabad, Maharashtra, India

Article Received on: 09/04/2011 Revised on: 18/05/2011 Approved for publication: 10/06/2011

*Prof. Vandana P. Patil, Yash Institute of Pharmacy, Aurangabad-431134, Maharashtra, India.

E-mail: vandana2609@gmail.com

ABSTRACT

Ultra performance liquid chromatography (UPLC) takes advantage of technological strides made in particle chemistry performance, system optimization, detector design and data processing and control. Using sub-2 μm particles and mobile phases at high linear velocities and instrumentation that operates at higher pressures than those used in HPLC, dramatic increases in resolution, sensitivity and speed of analysis can be obtained. This new category of analytical separation science retains the practicality and principles of HPLC while creating a step function improvement in chromatographic performance. This review introduces the theory of UPLC and summarizes some of the most recent work in the field.

KEYWORDS: UPLC, HPLC, Resolution, Sensitivity.

INTRODUCTION

UPLC refers to Ultra Performance Liquid Chromatography. It improves in three areas: chromatographic resolution, speed and sensitivity analysis. It uses fine particles and saves time and reduces solvent consumption. UPLC is comes from HPLC. HPLC has been the evolution of the packing materials used to effect the separation. An underlying principle of HPLC dictates that as column packing particle size decreases, efficiency and thus resolution also increases. As particle size decreases to less than 2.5 μm, there is a significant gain in efficiency and it doesn't diminish at increased linear velocities or flow rates according to the common Van Deemter equation. By using smaller particles, speed and peak capacity (number of peaks resolved per unit time) can be extended to new limits which is known as Ultra Performance. The classic separation method is of HPLC (High Performance Liquid Chromatography) with many advantages like robustness, ease of use, good selectivity and adjustable sensitivity. It's main limitation is the lack of efficiency compared to gas chromatography or the capillary electrophoresis due to low diffusion coefficients in liquid phase, involving slow diffusion of analytes in the stationary phase.^{1,2} The Van Deemter equation shows that efficiency increases with the use of smaller size particles but this leads to a rapid increase in back pressure, while most of the HPLC system can

operate only up to 400 bar.³ That is why short columns filled with particles of about 2 μm are used with these systems, to accelerate the analysis without loss of efficiency, while maintaining an acceptable loss of load. To improve the efficiency of HPLC separations, the following can be done:-

- i) Work at higher temperatures
- ii) Use of monolithic columns

USE OF THE UPLC SYSTEM

Elevated-temperature chromatography also allows for high flow rates by lowering the viscosity of the mobile phase, which significantly reduces the column back pressure. Monolithic columns contain a polymerized porous support structure that provides lower flow resistances than conventional particle-packed columns.^{4,5,6}

PRINCIPLE

The UPLC is based on the principal of use of stationary phase consisting of particles less than 2 μm (while HPLC columns are typically filled with particles of 3 to 5 μm). The underlying principles of this evolution are governed by the van Deemter equation, which is an empirical formula that describes the relationship between linear velocity (flow rate) and plate height (HETP or column efficiency). The Van Deemter curve, governed by an equation with three components shows that the usable flow range for a good efficiency with small diameter particles is much greater than for larger

diameters.^{7,8}

$$H=A+B/v+Cv$$

Where A, B and C are constants and v is the linear velocity, the carrier gas flow rate. The A term is independent of velocity and represents "eddy" mixing. It is smallest when the packed column particles are small and uniform. The B term represents axial diffusion or the natural diffusion tendency of molecules. This effect is diminished at high flow rates and so this term is divided by v . The C term is due to kinetic resistance to equilibrium in the separation process. The kinetic resistance is the time lag involved in moving from the gas phase to the packing stationary phase and back again. The greater the flow of gas, the more a molecule on the packing tends to lag behind molecules in the mobile phase. Thus this term is proportional to v . Therefore it is possible to increase throughput and thus the speed of analysis without affecting the chromatographic performance. The advent of UPLC has demanded the development of a new instrumental system for liquid chromatography, which can take advantage of the separation performance (by reducing dead volumes) and consistent with the pressures (about 8000 to 15,000 PSI, compared with 2500 to 5000 PSI in HPLC). Efficiency is proportional to column length and inversely proportional to the particle size¹⁸. Therefore, the column can be shortened by the same factor as the particle size without loss of resolution. Efficiency is three times greater with 1.7 μm particles compared to 5 μm particles and two times greater compared to 3.5 μm particles. Resolution is 70% higher than with 5 μm particles and 40% higher than with 3.5 μm particles. High speed is obtained because column length with 1.7 μm particles can be reduced by a factor of 3 compared to 5 μm particles for the same efficiency and flow rate can be three times higher. The application of UPLC resulted in the detection of additional drug metabolites, superior separation and improved spectral quality.^{9,10}

SMALL PARTICLE CHEMISTRY

The promises of the van Deemter equation cannot be fulfilled without smaller particles than those traditionally used in HPLC. The design and development of sub-2 mm particles is a significant challenge and researchers have been active in this area for some time to capitalize on their advantages. Although high efficiency, non-porous 1.5 mm particles are commercially available, they suffer from poor loading capacity and retention due to low surface area. To maintain retention and capacity similar to HPLC, UPLC must use novel porous particles that can withstand high pressures. Silica based particles have good mechanical strength, but can suffer from a number

of disadvantages, which include a limited pH range and tailing of basic analytes. Polymeric columns can overcome pH limitations, but they have their own issues, including low efficiencies and limited capacities. In 2000, generation hybrid chemistry that took advantage of the best of both the silica and polymeric column worlds was introduced. Produced using a classical sol-gel synthesis that incorporates carbon in the form of methyl groups, these columns are mechanically strong with high efficiency and operate over an extended pH range. But, in order to provide the kind of enhanced mechanical stability required for UPLC, a second generation bridged ethane hybrid (BEH) technology was developed. These 1.7 mm particles derive their enhanced mechanical stability by bridging the methyl groups in the silica matrix.

Packing 1.7 mm particles into reproducible and rugged columns was also a challenge that needed to be overcome. Requirements include a smoother interior surface of the column hardware, and re-designing the end frits to retain the small particles and resist clogging. Packed bed uniformity is also critical, especially if shorter columns are to maintain resolution while accomplishing the goal of faster separations. In addition, at high pressures, frictional heating of the mobile phase can be quite significant and must be considered. With column diameters typically used in HPLC (3.0 to 4.6 mm), a consequence of frictional heating is the loss of performance due to temperature induced non uniform. To minimize the effects of frictional heating, smaller diameter columns (1–2.1 mm) are typically used for UPLC.¹¹

INSTRUMENTATION

Sample injection

In UPLC, sample introduction is critical. Conventional injection valves, either automated or manual, are not designed and hardened to work at extreme pressure. To protect the column from extreme pressure fluctuations, the injection process must be relatively pulse-free and the swept volume of the device also needs to be minimal to reduce potential band spreading. A fast injection cycle time is needed to fully capitalize on the speed afforded by UPLC, which in turn requires a high sample capacity. Low volume injections with minimal carryover are also required to increase sensitivity. There are also direct injection approaches for biological samples.^{12,13}

UPLC Column

Resolution is increased in a 1.7 μm particle packed column because efficiency is better. Separation of the components of a sample requires a bonded phase that provides both retention and selectivity. Four bonded phases are available for UPLC separations: ACQUITY

UPLCT M BEH C18 and C8 (straight chain alkyl columns), ACQUITY UPLC BEH Shield RP18 (embedded polar group column) and ACQUITY UPLC BEH Phenyl (phenyl group tethered to the silyl functionality with a C6 alkyl). Each column chemistry provides a different combination of hydrophobicity, silanol activity, hydrolytic stability and chemical interaction with analytes.

ACQUITY UPLC BEH C18 and C8 columns are considered the universal columns of choice for most UPLC separations by providing the widest pH range. They incorporate trifunctional ligand bonding chemistries which produce superior low pH stability. This low pH stability is combined with the high pH stability of the 1.7 μm BEH particle to deliver the widest usable pH operating range. ACQUITY UPLC BEH Shield RP18 columns are designed to provide selectivities that complement the ACQUITY UPLC BEH C18 and C8 phases. ACQUITY UPLC BEH Phenyl columns utilize a trifunctional C6 alkyl tether between the phenyl ring and the silyl functionality. This ligand, combined with the same proprietary end capping processes as the ACQUITY UPLC BEH C18 and C8 columns, provides long column lifetimes and excellent peak shape. This unique combination of ligand and end capping on the 1.7 μm BEH particle creates a new dimension in selectivity allowing a quick match to the existing HPLC column. An internal diameter (ID) of 2.1 mm column is used. For maximum resolution, choose a 100 mm length and for faster analysis, and higher sample throughput, choose 50 mm column. Half-height peak widths of less than one second are obtained with 1.7 μm particles, which gives significant challenges for the detector. In order to integrate an analyte peak accurately and reproducibly, the detector sampling rate must be high enough to capture enough data points across the peak. The detector cell must have minimal dispersion (volume) to preserve separation efficiency. Conceptually, the sensitivity increase for UPLC detection should be 2-3 times higher than HPLC separations, depending on the detection technique. MS detection is significantly enhanced by UPLC; an increased peak concentration with reduced chromatographic dispersion at lower flow rates promotes increased source ionization efficiencies. The ACQUITY UPLC System consists of a binary solvent manager, sample manager including the column heater, detector, and optional sample organizer. The binary solvent manager uses two individual serial flow pumps to deliver a parallel binary gradient. There are built-in solvent select valves to choose from up to four solvents. There is a 15,000-psi pressure limit (about 1000 bar) to

take full advantage of the sub-2 μm particles. The sample manager also incorporates several technology advancements. Using pressure assisted sample introduction, low dispersion is maintained through the injection process, and a series of pressures transducers facilitate self-monitoring and diagnostics. It uses needle-in-needle sampling for improved ruggedness and needle calibration sensor increases accuracy. Injection cycle time is 25 seconds without a wash and 60 sec with a dual wash used to further decrease carry over. A variety of micro titer plate formats (deep well, mid height, or vials) can also be accommodated in a thermostatically controlled environment. Using the optional sample organizer, the sample manager can inject from up to 22 micro titer plates. The sample manager also controls the column heater. Column temperatures up to 65°C can be attained. To minimize sample dispersion, a "pivot out" design allows the column outlet to be placed in closer proximity to the source inlet of an MS detector.¹⁴

For UPLC detection, the tunable UV/Visible detector is used which includes new electronics and firmware to support Ethernet communications at the high data rates. Conventional absorbance-based optical detectors are concentration sensitive detectors, and for UPLC use, the flow cell volume would have to be reduced in standard UV/Visible detectors to maintain concentration and signal. According to Beer's Law, smaller volume conventional flow cells would also reduce the path length upon which the signal strength depends. A reduction in cross-section means the light path is reduced, and transmission drops with increasing noise. Therefore, if a conventional HPLC flow cell were used, UPLC sensitivity would be compromised. The ACQUITY Tunable UV/Visible detector cell consists of a light guided flow cell equivalent to an optical fiber. Light is efficiently transferred down the flow cell in an internal reflectance mode that still maintains a 10mm flow cell path length with a volume of only 500 μl . Tubing and connections in the system are efficiently routed to maintain low dispersion and to take advantage of leak detectors that interact with the software to alert the user to potential problems.¹⁵

ADVANTAGES

- Decreases run time and increases sensitivity.
- Provides the selectivity, sensitivity and dynamic range of LC analysis.
- Maintaining resolution performance.
- Expands scope of Multiresidue Methods.
- UPLC's fast resolving power quickly quantifies related and unrelated compounds.
- Faster analysis through the use of a novel separation material of very fine particle size.

- Operation cost is reduced.
- Less solvent consumption.
- Reduces process cycle times, so that more product can be produced with existing resources.
- Increases sample throughput and enables manufacturers to produce more material that consistently meet or exceeds the product specifications, potentially eliminating variability, failed batches or the need to re-work material.
- Delivers real-time analysis in step with manufacturing processes.
- Assures end-product quality, including final release testing.¹⁷

DISADVANTAGES

Due to increased pressure requires more maintenance and reduces the life of the columns of this type. So far performance similar or even higher has been demonstrated by using stationary phases of size around 2 μm without the adverse effects of high pressure. In addition, the phases of less than 2 μm are generally non-regenerable and thus have limited use.

APPLICATIONS OF UPLC

Analysis of amino acids

UPLC used for accurate, reliable and reproducible analysis of amino acids in the area of protein characterization, cell culture monitoring and nutritional analysis of foods.

Analysis of natural products and traditional herbal medicine

UPLC is widely used for analysis of natural products and herbal medicines. The main purpose of this is to analyze drug samples arise from the complexity of the matrix and variability from sample to sample. Purification and qualitative and quantitative chromatography and mass spectrometry are being applied to determine active drug candidates and to characterize the efficacy of their candidate remedies. UPLC provides high-quality separations and detection capabilities to identify active compounds in highly complex samples that results from natural products and traditional herbal medicines.

Analysis of Levofloxacin in human plasma.

Identification of Metabolite

Biotransformation of new chemical entities (NCE) is necessary for drug discovery. When a compound reaches the development stage, metabolite identification becomes a regulated process. It is of the utmost importance for lab to successfully detect and identify all circulating metabolites of a candidate drug. Discovery studies are generally carried out in vitro to identify major metabolites so that metabolic weak spots on the drug candidate molecule can be recognized and protected by changing the compound structure.

Study of Metabonomics / Metabolomics

Metabonomics studies are carried out in labs to accelerate the development of new medicines. The ability to compare and contrast large sample groups provides insight into the biochemical changes that occur when a biological system is exposed to a new chemical entity (NCE). Metabonomics provides a rapid and robust method for detecting these changes improves understanding of potential toxicity and allows monitoring the efficacy.

ADME Screening

ADME studies measure physical and biochemical properties absorption, distribution, metabolism, elimination, and toxicity of drugs where such compounds exhibit activity against the target disease. A significant number of candidate medicines fall out of the development process due to toxicity. If toxic reactions or any side effect occurs in the discovery/development process, then it becomes more costly. It is difficult to evaluate candidate drugs for possible toxicity, drug-drug interactions, inhibition, and/or induction of metabolizing enzymes in the body. Failure to properly identify these potential toxic events can cause a compound to be withdrawn from the market. The high resolution of UPLC enables accurate detection and integration of peaks in complex matrices and extra sensitivity allows peak detection for samples generated by lower concentration incubations and sample pooling.

Bioanalysis/ Bioequivalence Studies

For pharmacokinetic, toxicity, and bioequivalence studies, quantization of a drug in biological samples is an important part of development programs. The drugs are generally of low molecular weight and are tested during both preclinical and clinical studies. Several biological matrices are used for quantitative bioanalysis, the most common being blood, plasma and urine.

Dissolution Testing

For quality control and release in drug manufacturing, dissolution testing is essential in the formulation, development and production process. In sustained-release dosage formulations, testing higher potency drugs is particularly important where dissolution can be the rate-limiting step in medicine delivery. The dissolution profile is used to demonstrate reliability and batch-to-batch uniformity of the active ingredient. Additionally, newer and more potent formulations require increased analytical sensitivity.

Forced Degradation Studies

One of the most important factors that impacts the quality and safety of pharmaceuticals is chemical stability. The FDA and ICH require stability testing data to understand

how the quality of an API (active pharmaceutical ingredient) or a drug product changes with time under the influence of environmental factors such as heat, light, pressure and moisture or humidity. Knowledge of these stability characteristics defines storage conditions and shelf life, the selection of proper formulations and protective packaging and is required for regulatory documentation. Forced degradation or stress testing is carried out under even harsher conditions than those used for accelerated stability testing.

Manufacturing/ QA/ QC

Identity, purity, quality, safety and efficacy are the important factors to be considered while manufacturing a drug product. The successful production of quality pharmaceutical products requires that raw materials meet purity specifications. That manufacturing processes proceed as designed. That final pharmaceutical product meets, and hopefully exceeds, defined release specifications. Continued monitoring of material stability is also a component of quality assurance and control. UPLC is used for the highly regulated, quantitative analyses performed in QA/QC laboratories.

Method Development / Validation

According to FDA, validation is defined as establishing documented evidence that provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes. Method development and validation is a time-consuming and complicated process: labs need to evaluate multiple combinations of mobile phase, pH, temperature, column chemistries and gradient profiles to arrive at a robust, reliable separation for every activity. UPLC help in critical laboratory function by increasing efficiency, reducing costs and improving opportunities for business.

Impurity Profiling

For the drug development and formulation process, profiling, detecting, and quantifying drug substances and their impurities in raw materials and final product testing is an essential part. Impurity profiling requires high-resolution chromatography capable of reliably and reproducibly separating and detecting all of the known impurities of the active compound. Also critical is the ability to accurately measure low-level impurities at the same time as the higher concentration active pharmaceutical component. UPLC System and Columns specifically address high-throughput analysis requirements while maintaining high peak resolution. UPLC also involves the latest peak detection algorithms and custom calculations to optimize data processing and reporting. It also confidently detects impurities in compounds even at trace levels.

Compound Library Maintenance

Confirming the identity and purity of a candidate pharmaceutical is critical to effectively screening chemical libraries that contain vast types of small molecules across a range of biological targets. Chemists need to be sure they have synthesized the expected compound. In this high-throughput screening environment, the ability to obtain information in multiple MS and UV detection modes in a single injection is invaluable. Combining fast analysis with open-access software delivers the power of LC/MS to chemists who are not analytical instrumentation specialists. A single complete system enables them thoroughly screen a compound, from sample introduction to end results.

Open Access

Maximum efficiency is essential for analytical laboratories that are constantly challenged to increase throughput and deliver results to research chemists in pharmaceutical discovery. UPLC and UPLC/MS systems and software enable versatile and open operation for medicinal chemistry labs, with easy-to-use instruments, a user-friendly software interface, and fast, robust analyses using UV or MS for nominal and exact mass measurements.^{18,19,20}

CONCLUSION

UPLC increases productivity in both chemistry and instrumentation by providing more information per unit of work as it gives increased resolution, speed, and sensitivity for liquid chromatography. The main advantage is a reduction of analysis time, which also meant reduced solvent consumption. The time spent optimizing new methods can also be greatly reduced. The time needed for column equilibration while using gradient elution and during method validation is much shorter. Sensitivity can be compared by studying the peak width at half height. It was found that the sensitivity of UPLC was much higher than that of conventional HPLC.

REFERENCES

1. Kondawar M. S; Ultra Performance Liquid Chromatography: A Faster and Sensitive Method over HPLC; Available from: <http://www.pharmainfo.net/>
2. Zhu C, Goodall DM, Wren SAC. Elevated Temperature HPLC: Principles and Applications to Small Molecules and Biomolecules. LCGC. 2005; 23(1):54-72.
3. Michael E Swartz; UPLC: An introduction and review; Journal of Liquid Chromatography & Related Technologies. 2005; 28: 1253-1263.
4. Wu N, Dempsey J, Yehl PM, Dovletoglu A, Ellison A and Wyvratt J; Practical aspects of fast HPLC separations for pharmaceutical process development using Monolithic Columns ; Anal. Chem. Acta. 2004; 523:149-156.
5. Tanaka N, Kobayashi H, Nakanishi K, Minakuchi H and Ishizuka N; Monolithic columns – a new type of chromatographic support for liquid chromatography; Anal.

- Chem. 2001; 73:420A–429A.
- Jerkovich AD, Mellors JS and Jorgenson J W; Uplc: An Sensitive And High Throughput Analysis Over Hplc Mpy-322; LCGC.2003; 21(7):600–610.
 - Mac Nair JE, Lewis KC and Jorgenson JW; Ultrahigh-Pressure Reversed-Phase Liquid Chromatography in Packed Capillary Columns. Anal Chem. 1997; 69:983-989.
 - Mac Nair JE, Patel KD and Jorgenson JW; Ultrahigh-Pressure Reversed-Phase Capillary Liquid Chromatography: Isocratic and Gradient Elution Using Columns Packed with 1.0 mm Particles. Anal Chem. 1999; 71:700-708.
 - Wu N, Lippert JA and Lee ML; Practical aspects of ultra high pressure capillary liquid chromatography; J. Chromatogr. A. 2001; A 911:1–12.
 - Patel KN and *et al*; A Review: Ultra Performance Liquid Chromatography; International journal of biomedical research and analysis. July-Aug 2010; volume 1 , issue 1:1-4.
 - Swartz ME; Ultra performance liquid chromatography: Tomorrow's HPLC technology today. Lab Plus Int. 2004; 18(3): 6-9.
 - Mc Loughlin DA, Olah TV, and Gilbert JD; A direct technique for the simultaneous determination of 10 drug candidates in plasma by liquid chromatography-atmospheric pressure chemical ionization mass spectrometry interfaced to a prospekt solid-phase extraction system; J. Pharm. Biomed. Anal. 1997; 15: 1893–1901.
 - Swartz ME; Ultra Performance Liquid Chromatography (UPLC): An Introduction. Separation Science Re-Defined. 2005; LCGC Supplement: 11.
 - Swartz ME; Ultra Performance Liquid Chromatography (UPLC): An Introduction, Separation Science Re-Defined. 2005; LCGC Supplement: 13.
 - Uttam Singh Baghel and *et al*; Ultra Performance Liquid Chromatography: A Chromatography Technique; International Journal of Pharmaceutical Quality Assurance 2010; 2(1): 19-25.
 - Gaikwad PV and *et al*; Ultra Performance Liquid Chromatography: A recent novel development in HPLC; Pharmacie Globale (IJCP). 2010; 2:08.
 - Swartz ME. UPLC: An Introduction and Review. J. Liq. Chrom. 2005; 28:1253-1263.
 - Sherma Joseph, D John, Larkin and Frances H; UPLC: Ultra Performance Liquid Chromatography; Journal of AOAC International. 2005; May 1.
 - Sikk P and *et al*; Ultra performance liquid chromatography analysis of adenine nucleotide and creatine derivative for kinetic studies; Proceedings of the Estonian Academy of Sciences. 2009; 58(2):122-131.
 - Park DJ and *et al*; An improved UPLC method for analysis of Levofloxacin in human plasma; Chromatographia. 2008; 68, August (No. 3/4): 187-192.

Table 1: Comparison between UPLC and HPLC¹⁶

Characteristics	HPLC Assay	UPLC Assay
Column	150 X 3.2 mm	150 X 2.1 mm
Particle size	3 to 5µm	Less than 2µm
Flow rate	3.0 ml / min	0.6 ml / min
Needle wash	Methanol	Methanol
Injection volume	5µL (Std.In 100 % MeOH)	2µL(Std.In 100 % MeOH)
Column temperature	30 °C	65 °C
Maximum backpressure	35-40 MPa	103.5 MPa
Gradient (time in min) ACN:H ₂ O	T0 (25:75), T6.5 (25:75), T7.5 (95:5), T9 (25:75), T10 (25:75)	T0 (36:64), T1.1 (95:5), T1.3 (36:64)
Total run time	10min	1.5min
Total solvent consumption (including 0.5 min of delay time in between injections)	Acetonitrile: 10.5 ml Water: 21.0 ml	Acetonitrile: 0.53 ml Water: 0.66 ml
Plate count	2000	7500
USP resolution	3.2	3.4
Delay volume	750 µl	110 µl